Human Adenovirus B7d–Associated Urethritis after Suspected Sexual Transmission, Japan

Nozomu Hanaoka, Shin Ito, Naomi Nojiri, Masami Konagaya, Mitsuru Yasuda, Takashi Deguchi, Tsuguto Fujimoto

Outbreaks of acute respiratory disease associated with human adenovirus (HAdV) B7d have been reported, including fatal cases in the United States. In 2018, we detected HAdV-B7d in a patient with urethritis, probably transmitted through sexual contact. Infectious HAdV-B7d was excreted in urine and gargle for ≥10 days after the disappearance of symptoms.

Human adenoviruses (HAdVs) are DNA viruses that can cause respiratory diseases, conjunctivitis, and gastroenteritis (*1*). Seven species (A–G) and ≥100 types have been recognized so far. Among them, HAdV-E4, HAdV-B7, and HAdV-B14 cause severe acute respiratory illness, including severe acute respiratory distress syndrome (*2*). HAdV-B7d, a genome type of HAdV-B7, was originally designated in 1986 using restriction analysis (*3*) and classified as genotype B7d on the basis of complete genome analysis in 2013 (*4*). HAdV-B7d was first reported in China in 1980 (*3*); by the 1990s, HAdV-B7d was the primary circulating genome type in China, but then it was not detected during 1990–2009. In 2011, HAdV-B7d was prevalent throughout Asia, and outbreaks of infant pneumonia related to HAdV-B7d were reported in China (*5*–*7*).

In Japan, routine national surveillance for HAdVs is conducted for epidemic keratoconjunctivitis, pharyngeal conjunctival fever, and infectious gastroenteritis, and reported in the *Infectious Diseases Weekly Report* (*8*). Outbreaks of HAdV-B7d, including 2 fatal cases, were observed in Japan during 1995–1996 (*9*), after which it was rarely detected until the occurrence of the case we describe in 2018 (*10*).

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In 2014, HAdV-B7d was detected in Oregon and Illinois, USA (*11*,*12*). During 2016–2017, a total of 12 cases were reported, including 3 patients in a residential rehabilitation center, 7 college students, and 2 patients at a tertiary care hospital. Four of those 12 case-patients died; all 4 were in 3 adjacent New Jersey counties and had underlying conditions (*2*,*13*,*14*). In 2018, an HAdV-B7d outbreak at multiple facilities in several US regions resulted in the deaths of 11 infants at rehabilitation center in New Jersey and an 18-year-old freshman at the University of Maryland (*15*). In summary, HAdV-B7d transmission occurred in community and congregate settings throughout the United States, resulting in severe illness and death in some patients with underlying conditions. HAdV-B7d has been more commonly associated with severe respiratory disease and has a higher mortality rate than other HAdV types (*6*,*12*). Therefore, clinicians and public health facilitators should consider HAdV-B7d in patients with severe respiratory infections.

The Study

Since 2013, we have focused on HAdV-associated urethritis and performed pathogen screening from the urine of all-male patients with acute urethritis at iClinic in Sendai City, Miyagi Prefecture, Japan; all patients gave informed consent (reference *16* in Appendix, https://wwwnc.cdc.gov/EID/26/10/19-1538-App1.pdf). Recently, several papers have reported that HAdVs are ranked the third- or fourth-highest causative agents of nonchlamydial, nongonococcal urethritis (reference *17* in Appendix). Furthermore, HAdVs most commonly associated with urethritis are those that cause epidemic keratoconjunctivitis, which are types D37, D56, and D64 (references *16*,*18* in Appendix).

In July 2018, a case of male urethral inflammation associated with HAdV-B7d was detected in a 22-year-old heterosexual man. He was unmarried and did not

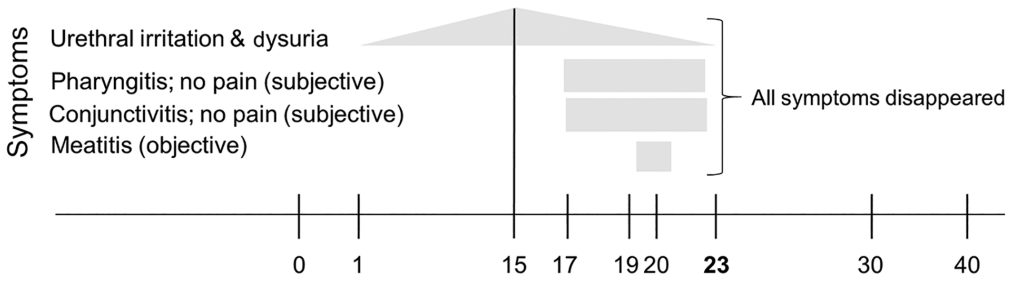

Figure 1. Clinical course and laboratory test results for patient with HAdV B7d–associated urethritis, Japan. HAdV, human adeovirus.

Day 0 Sexual activity; the day of putative infection of pathogens related to urethritis.

Day 1 Urethral irritation and dysuria appeared. (Subjective symptoms)

Day 15 Maximum dysuria score: Numerical Rating Scale (NRS) 5 and Visual Analogue Scale (VAS) 4.8

Day 17 Pharyngitis, conjunctival hyperemia and eye discharge without pain and foreign body sensation were appeared.

Day 19 Visit ophthalmic clinic and diagnosed by an eye medicine as not adenoviral conjunctivitis
Adenoviral immunochromato Kit performed with negative result
Prescribed Odemel eye drop and levofloxacin eye drop

Day 20 First visit to iClinic
Symptoms
Dysuria score; NRS4 and VAS3.6. No urethral irritation, discharge and pollakisuria.
Signs
Inflammation around external urethral meatus. Granular hypospadias. Low- urethral serous discharge.
Both testicles and epididymis were normal. Not observed both sides of groin pain or swelling.
Hyperemia of both bulbar conjunctiva and palpebral conjunctiva

Urethral discharge, first void urine (FVU), gargle and eye swab of left lower eyelid were collected
Microscopy
>20 leukocytes / HPF, mononucleosis <10%, not observed phagocytosis of diplococcus.
1123.7 leukocytes /μL in FVU
Diagnosis
non-gonococcal urethritis
Treatment
Sitafloxacin Hydrate 200mg/ day for 7 days.

Detected and isolated pathogen
FVU: HAdV (8×10^3/mL)
Urethral discharge: Haemophilius parainfluenza
Gargle: HAdV (5×10^5/mL)
Eye swab of the left lower eyelid: HAdV (1×10^3/mL)

Day 23 All symptoms disappeared (subjective)

Day 30 Revisit to iClinic
No symptoms (subjective and objective)
Microscopy
15.0 /μL leukocytes /μL in FVU
Treatment
No

Detected and isolated pathogen
First void urine: HAdV (5×10^2/mL)
Gargle: HAdV (1×10^3/mL)
Eye swab of left lower eyelid: negative

Day 40 Third visit to iClinic
No symptoms (subjective and objective)

HAdV not detected or isolated

have a specific sexual partner. He had an unremarkable medical history, no history of sexually transmitted infections, and no record of traveling abroad. He claimed to have had 2 sexual encounters during his lifetime; the first was in 2016, but the second, in 2018, we considered to be the putative infection day (day 0) (Figure 1). He described it as a casual sexual encounter with a previously unknown woman and reported the encounter to include protected vaginal intercourse, cunnilingus, and unprotected oral intercourse. The patient denied insertive or receptive anal intercourse as well as mouth-to-mouth kissing. Urethral irritation and dysuria appeared on day 1 and continued to develop gradually (Figure 1); these symptoms might be

caused by mechanical irritation. On day 15, the patient experienced the most pain from his symptoms, reporting a numerical rating scale score of 5/10 and a visual analog scale score of 4.8/10. These scales are subjective scores of pain (reference *19* in Appendix). Pharyngitis and conjunctivitis appeared on day 17. On day 19, he visited an ophthalmic clinic for confirmation of conjunctivitis; however, it was not considered to be adenoviral conjunctivitis because an adenoviral immunochromatographic kit produced a negative result. Fluorometholone and levofloxacin eye drops were prescribed. Because his urethritis symptoms were severe, he visited a sexually transmitted diseases clinic (Sendai city, Miyagi prefecture, Japan) on day 20. The patient reported no fever, chills, or malaise; on the basis of his symptoms and results of a physical examination (Figure 1), we diagnosed nongonococcal urethritis. Because we could not exclude the possibility of bacterial infection, we prescribed sitafloxacin hydrate (200 mg/d for 7 days). Pathogen screening at the first visit to the clinic detected *Haemophilus parainfluenzae* bacteria from urethral discharge, but the clinical significance was unclear.

We isolated HAdV in A549 cells from first-void urine, throat gargle, and eye discharge fluid by a previously described method (reference *16* in Appendix). No other pathogens were identified. Sequences of the HAdV hexon, fiber, and penton open reading frames obtained by Sanger sequencing from all 3 specimen sources were identical. The full genome sequence was obtained from the urine isolate (designated strain 293) (Appendix Tables 1, 2) and deposited in GenBank (accession no. LC530212). We also performed a BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) as previously described (*2*) with reference sequences of HAdV type 7 (Appendix Figure 1, Figure 2). On the basis of the phylogenetic tree analysis of whole-genome sequences, we classified the isolated HAdV-B7 strain into the same cluster as HAdV-B7d. In addition, we performed an in silico analysis of the genome using Restriction Analyzer software (http://www.molbiotools.com/restrictionanalyzer.html) by comparing the patterns of the 293 isolate with a reference HAdV-7d sequence and the following enzymes: *Bam*HI, BclI, BstEII, *Hpa*I, and *Sma*I (Appendix Figure 2). After these analyses, we identified the 293 isolate as HAdV-7d. On

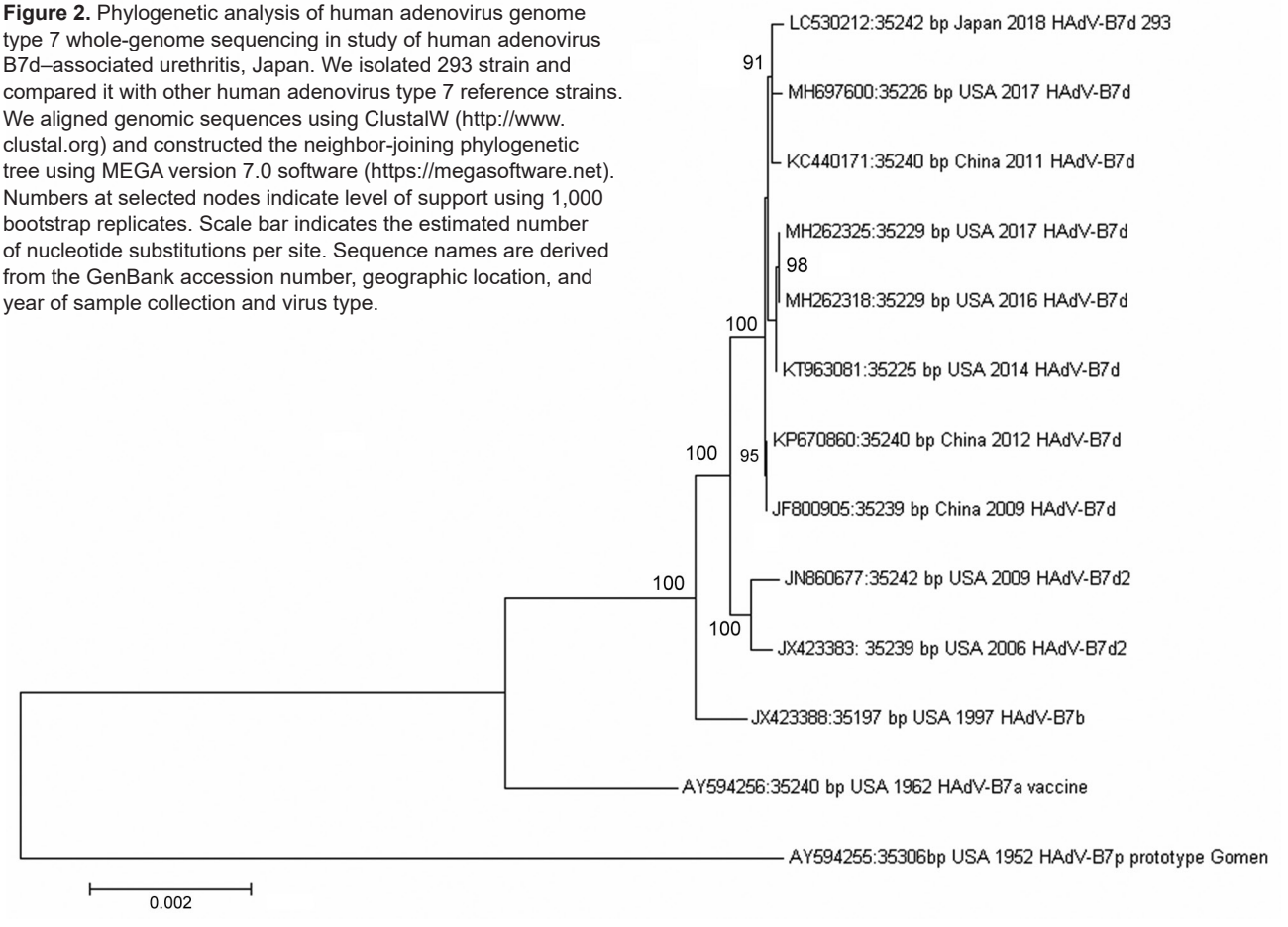

Figure 2. Phylogenetic analysis of human adenovirus genome type 7 whole-genome sequencing in study of human adenovirus B7d–associated urethritis, Japan. We isolated 293 strain and compared it with other human adenovirus type 7 reference strains. We aligned genomic sequences using ClustalW (http://www.clustal.org) and constructed the neighbor-joining phylogenetic tree using MEGA version 7.0 software (https://megasoftware.net). Numbers at selected nodes indicate level of support using 1,000 bootstrap replicates. Scale bar indicates the estimated number of nucleotide substitutions per site. Sequence names are derived from the GenBank accession number, geographic location, and year of sample collection and virus type.

the basis of these results, we concluded that the patient acquired the HAdV-B7d infection, which caused urethritis, conjunctivitis, and pharyngitis, during sexual intercourse. All symptoms disappeared by day 23. When the patient revisited the clinic on day 30, he had no urethral symptoms. We detected HAdV-B7d strains isolated from first-void urine and gargle but not from eye discharge; no other pathogens tested in this study were detected. On day 40, the patient's third visit to the clinic, no pathogens were detected. Approximately 2 months later, no symptoms were observed, and we confirmed a good prognosis.

Conclusions

HAdVs infect mucous membranes and can infect the urethra. Documented cases of HAdV urethritis are most often associated with certain species D HAdVs that cause epidemic keratoconjuctivitis (references *16*–*18* in Appendix). Our finding of HAdV-B7d, a virus more commonly associated with acute respiratory infections, in this patient was unexpected. Although we identified *H. parainfluenza* from urethral discharge at the first clinic visit, we suspect that it may have contributed to but not caused the patient's primary symptoms. The isolated HAdV-B7d strains in this study were excreted in urine and gargle for ≥1 week after all symptoms had disappeared (Figure 1), which suggests that HAdV-B7d infection may cause urethritis and involve viral shedding into urine.

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Appendix

Additional References

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Appendix Table 1. Primer name and sequence used in study of human adenovirus B7d–associated urethritis, Japan.

Name	Sequence
Ad7_5'-1	TCTATTTAATATACCTTATAGATG
Ad7_5'-2	ACTCTTGAGTGCCAGCGAGAAGAG
Ad7_5'-3	AAGCTGCCAGCGATGTTTTTAAGTTGG
Ad7_5'-4	TAAAATTATGTCAGCTGCTGAGTG
Ad7_5'-5	ACAATCTTAGATTACTGGCCAGTGC
Ad7_5'-6	TAATAGATACACAAGATAAAGCAG
Ad7_5'-7	TCTTGCTACTGTGCATATCGTTTCAC
Ad7_5'-8	AGTATTTAGCCCTTATCTGACGGGC
Ad7_5'-9	TTGAGAACTCGGTGGATCTTTTCCAG
Ad7_5'-10	TCTGAAACATCATAGTTATGCTCC
Ad7_5'-11	AAAGAGTCTGTTGCAAGAGCTCGAGCC
Ad7_5'-12	AGGTCAGATCCGGCTCATCGGGGTC
Ad7_5'-13	TAGGAACGAGGAGGATTTGATATTGAC
Ad7_5'-14	TCTCGAACTGCCAGCGCGCGCTCATAGG
Ad7_5'-15	AGTACTCTTCGAGGGGAAACCCGTC
Ad7_5'-16	AGTGTTCGAGGGCCCATTCGTGCACG
Ad7_5'-17	TGACTTCCTTTGACGAGAAATTTCAG
Ad7_5'-18	TGGTACTGCGCCCTGAGAAGACTCG
Ad7_5'-19	AGAAATACATGATCCATCGTCTCAGC
Ad7_5'-20	ATCGTCTCAAGATCCACGGGATCTG
Ad7_5'-21	ATTGTGTAGGAGCAGTTGGCCATGAC
Ad7_5'-22	AGCCGCAGCTAACGTGGTACTGGC
Ad7_5'-23	ACTGGCGCGCCTGGGCGCACCATCG
Ad7_5'-24	TAACTACTGCGGCTGCAGCCGTCAGC
Ad7_5'-25	ATCAGCCCAGCTAGGGCACATGTGG
Ad7_5'-26	ACTCTGAGCGACGATCTGGGGGTG
Ad7_5'-27	AACCCCACCCACGAGAAGGTCCTGG

Name	Sequence
Ad7_5'-28	TGCTGCTGCTGCTGGTATCC
Ad7_5'-29	AGTACGACATGCCCGACCCCAATG
Ad7_5'-30	TTGGGTGGAAGAGGAGGGGGCAACC
Ad7_5'-31	ATGGGGCGGTCATCTGAAGACCATC
Ad7_5'-32	ATGTATGAAGATCTTGAAGGGGG
Ad7_5'-33	AATGAGCAAGCCGTGTACTCTCAGC
Ad7_5'-34	TGCTCGTAATTATACTCCTACTGCGC
Ad7_5'-35	AAAAGCCCCGCAAAATCAAGCGGGTC
Ad7_5'-36	AGCCTGAGGTCAAAGTAAGACCTATC
Ad7_5'-37	ATATGGCCCTCACTTGCCGCCTTCG
Ad7_5'-38	AAATAAGCTTAAGGAACAAAACTTCC
Ad7_5'-39	TCTGAACAGCATCGTGGGTCTGGGC
Ad7_5'-40	TAGCTTCAAGCCATATTCCGGCAC
Ad7_5'-41	AATTTGGAAACTCCAGACAGCCATG
Ad7_5'-42	TTAATATCCAAGCTAATCTTTGGAG
Ad7_5'-43	ACTCTTGAAGCCATGCTGCGCAACG
Ad7_5'-44	AAACTTCCAGCCTATGAGCAGGCAGG
Ad7_5'-45	AGCTGCAGCCATGTCATGCGGGTC
Ad7_5'-46	AATCTCCCCAAGTGCAGCCCACCC
Ad7_5'-47	TGTCTCACGCTTGCCAGCACGGTCGG
Ad7_5'-48	TGCATCCATGAGCCCACAGAGCGC
Ad7_5'-49	TGGTCTTTCTGGGCTTCTTCTTGGG
Ad7_5'-50	AAGAGATTGAGGCAGATGTCGAGCAGG
Ad7_5'-51	TTACCTGATATAGCTTCCTTGGAAGAG
Ad7_5'-52	ACCTTTAGACATGGCTTCGTGCGG
Ad7_5'-53	ATGGAGCCACTGCTACCTGTTCCGC
Ad7_5'-54	AGGATGTCCCATCGCCGAGGAAGC
Ad7_5'-55	ATTACTACCGTCACCTCCACAGCC
Ad7_5'-56	TTGGCAGCAGGCGCCTCCCAGGAC
Ad7_5'-57	TCTCCAGTTCGTGGAGGAGTTTACTCC
Ad7_5'-58	ACCAACCAGATCTTCCAGAAGACCC
Ad7_5'-59	ACTGCAGGTCCGTTGAAATTACAC
Ad7_5'-60	ATTTGGACCAAACTTGGAAGTGTTG
Ad7_5'-61	TTGGCTCAACATGTACACTACAAGG

Name	Sequence
Ad7_5'-62	TATAGAATTATGATATTGTTTCAATC
Ad7_5'-63	ACTTCTTAGGCTTATTTAAAACCATGC
Ad7_5'-64	AACTACGCATCCGCCAGCAGCAGG
Ad7_5'-65	TGTACCCACAATCTTCATGTCTTTC
Ad7_5'-66	TAATTCTAACACTAGTTAAAACTGG
Ad7_5'-67	ACGACTGACAAATAAAGTTTAACTTG
Ad7_5'-68	ACTTAGATTACTACAGTAGGTACAGC
Ad7_5'-69	ACGAACACAACTTACACTATGCATAG
Ad7_5'-70	TAGATCGCGCAGATGGCATCTATCG
Ad7_5'-71	TACAAGCGCAGACCTCCCCAATTGG
Ad7_5'-72	ATGCTTAATCTTAAGTATAGCAAAGCC
Ad7_5_P7900	TTGTACAGACGGCCGCAGTACTCGC
Ad7_3_P9070	TTTTCAACTTTGCCGTGGACTTCTAC
Ad7_3'-1	TCTATATAATATACCTTATAGATGG
Ad7_3'-2	ATACTTAAGATTAAGCATAATTATACC
Ad7_3'-3	TGTAGCGTCCCCTGCTATTGTTCCC
Ad7_3'-4	ATTTGAAGTACTGCGAGATCGTTTGG
Ad7_3'-5	TTCCTTGTTCTGCCAGCTTTACTGTTC
Ad7_3'-6	TCAGAATGCGTTGCTGCGCGCACC
Ad7_3'-7	TCTAATGTAGTAAAAGGTAAATGGAG
Ad7_3'-8	ATTCACTGGAGGCCATTATTTGACAG
Ad7_3'-9	TTTGACATCCCCTTTAAAGTATGGAG
Ad7_3'-10	TTTGCATTGGTGAATTTGGATGAC
Ad7_3'-11	ATTTTGATTGCGGTATTCGGGATGG
Ad7_3'-12	ATGTCAAAATTTAGTAATGCATCAC
Ad7_3'-13	ATAAGTTACAGCTGCAAGGCTAGTAATG
Ad7_3'-14	AACCTCTGAAGTAATTGGGGGCCC
Ad7_3'-15	ACTTAATAAGAACTCCACAGAGACG
Ad7_3'-16	TTGTAAAGTCCTGATTGAAGAAGCGG
Ad7_3'-17	TTCCGATGCCGCCCGAGCGGGGC
Ad7_3'-18	ATTTGGGGCTGATAGCTCCACATG
Ad7_3'-19	AAGTTCACACCGTGGTGGAAGAGCAG
Ad7_3'-20	AACCTTGTGTTGAGCTCCTCACCGG
Ad7_3'-21	AGAGGCACAAAGTCGGAGGGCAGCG

Name	Sequence
Ad7_3'-22	TAGTGCAGGTTCTCCTCTAGCTTGC
Ad7_3'-23	AGCTCCCGGACCCAAGTTGAGAAGGG
Ad7_3'-24	TTTCACATTATCCTGCGCCTGCATC
Ad7_3'-25	AGCATGTCCCTCTGCAAGACATCGGC
Ad7_3'-26	ATTATGAAGGCAAGGTGAAATGCC
Ad7_3'-27	TTCCAGTGTTGCAACCCAGTGTACCG
Ad7_3'-28	TTTATTGAACACGGTTTTACATGAC
Ad7_3'-29	AAGCAAGAGGCTTCTTATGTGGTGGC
Ad7_3'-30	AAAAAGGAGTACATGCGATCCTTG
Ad7_3'-31	TGAAGCGGTGTTGTGAGCCATGGG
Ad7_3'-32	TTTCCATAGCCAGATTGTTGCCTATGG
Ad7_3'-33	TTCCGTGTAAAGCACAATTTCAGGCG
Ad7_3'-34	TAATGTCAAAGAATGTGCTGGCCATG
Ad7_3'-35	AGACCCACGATGCTGTTCAGAGTAC
Ad7_3'-36	AAGCTTTGTTCCCATAGGTTTTTACGG
Ad7_3'-37	AGGCAGCGTCAACAGTCATTAAGTGG
Ad7_3'-38	ATTTTCTCCAGTACGTCTTCTAGCC
Ad7_3'-39	TTTCATCCTTCACCGGTGGACCGTAG
Ad7_3'-40	TCGGCGACCACCTGGTCGATCACATC
Ad7_3'-41	TAGAAACTCTTTGAGAAGACGGGC
Ad7_3'-42	TTTGAAACCCTCCTGGAATGGATGTC
Ad7_3'-43	TTCGTCAAAGTTGATGGTCTGGGTG
Ad7_3'-44	TCCAACACGCTGCTATCATCGGCAG
Ad7_3'-45	AAACCAGGTGGGGGCAGCCAGTGTG
Ad7_3'-46	TTGGGAGTCAGCAAGCTAGACACGGTC
Ad7_3'-47	AAAGGCGGTTGGCCTGGGGTTGCTG
Ad7_3'-48	AGAGTCATGCGCATGTAAAACCCATC
Ad7_3'-49	TCCTCGTCTTGCAGCACCCGTCTTCG
Ad7_3'-50	AGTAGTTACAGGAGCAGGAAGAGCC
Ad7_3'-51	TCTTCCAATTCCAGATCATAGGCGG
Ad7_3'-52	TGATCCGAGATTCGAACCGGGGTAC
Ad7_3'-53	TTTGTGGACTTTGACGACTTCCAAG
Ad7_3'-54	TTGCGCGGAGTACCTACGGGGCAATTG
Ad7_3'-55	TCGTCACCGAGCACATCGCCACCAC

Name	Sequence
Ad7_3'-56	AACCTACCCGCGCGCGGCGCCGAC
Ad7_3'-57	ATTCATGAGGTGCATCCCGTGAATCG
Ad7_3'-58	TGCTCTCACTGACCCTACAGATCTCAC
Ad7_3'-59	AAGGATCGCGAAGAATACCTTCTC
Ad7_3'-60	AGATGACCTTGGATGATCCCACCACC
Ad7_3'-61	AACTGACAACCTGAGTGCAGAGGTC
Ad7_3'-62	AAACCGTCTGCGCCTCCTGCGGTGCG
Ad7_3'-63	AAATGCCCATGGCTGACGGGCTGAAG
Ad7_3'-64	TATCATTATGGATGAGTGCATGGAG
Ad7_3'-65	TGATCGAGACCGATGGTCCAGGGC
Ad7_3'-66	AAGCGCTTCCACTCATGGCAGCTGC
Ad7_3'-67	ATATTGCAATGTCCACCAGCGCAGG
Ad7_3'-68	ATTCCCTGATATGTAGCATGC
Ad7_3'-69	TCATCCTGCGAGCCTTCCATGTTC
Ad7_3'-70	TACATGACGTCACATTAAATAAACAC
Ad7_3'-71	ATTCACTGCGGTATGGATGGACTGCTC
Ad7_3'-72	AACTCGTCAGGTTTAAATACCCTAGCG
Ad7_5_39_2	ATGCTTCGGAGTACCTGAGTCCGG
Ad7_5_40_2	ATATCAGCCAGAGCCTCAAGTTGG
Ad7_3_34_2	TTTCATTTGTTCCATCAATATCAG
Ad7_3_33_2	TTCAGTGTTTCTGTCCTGCAAGTC
Ad7_up_3_1*	TTTTAGCCGTTCACCCCACAGCC
Ad7_up_3_2*	AATTTTTACTTGCATCCGCC
Ad7_down_5_1*	TTTTTTAAATTACCTCATTTGC
Ad7_down_5_2*	AGGGAAAAGTACAGTTTCACTTCC

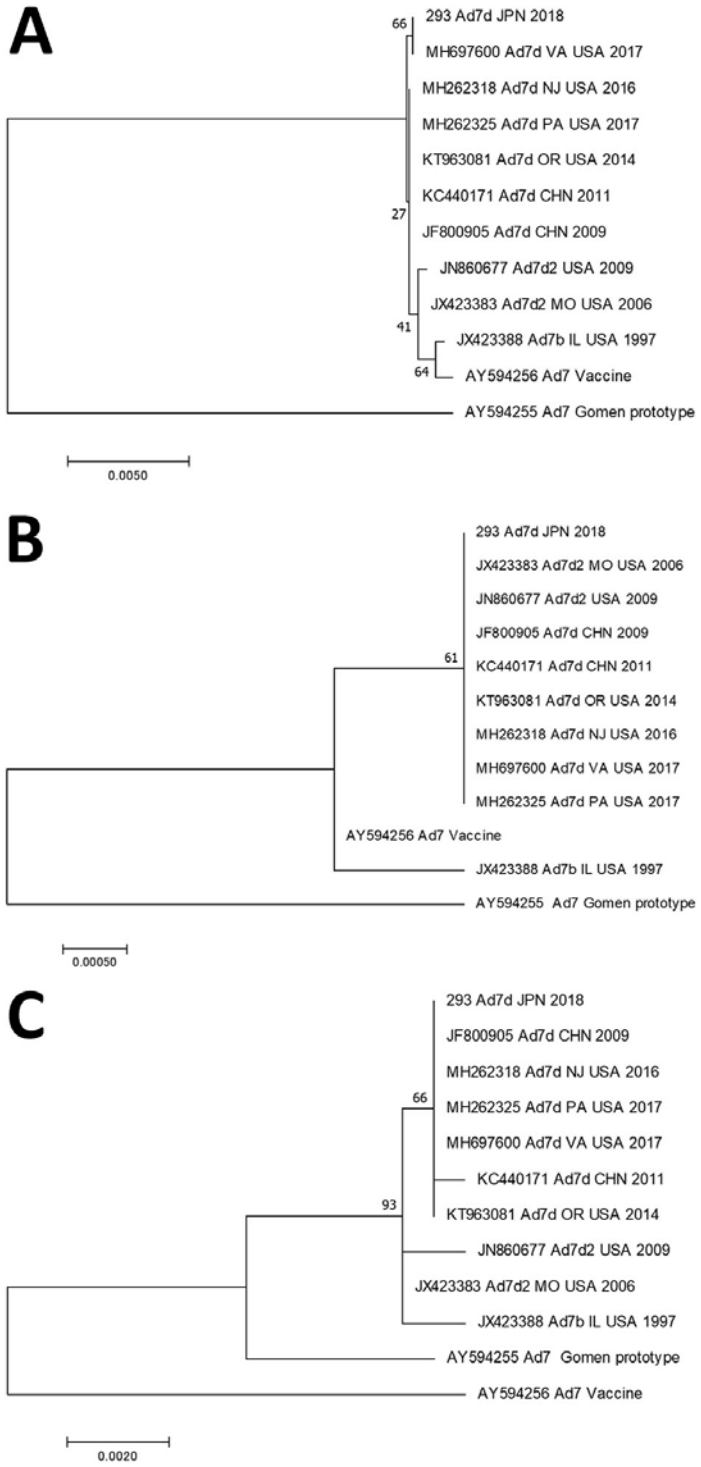
*5' and 3' end of 293 genome was determined by direct sequencing using Ad7_up3_1, 3_2, Ad7_down_5_1 and 5_2 with its genomic DNA.

Appendix Table 2. Primer set for PCR amplification and sequencing in study of human adenovirus B7d–associated urethritis, Japan*

Fragment	1-3500	3000-6500	5500-8000	6000-9500	7500-10000	9000-12500	12000-15500	15000-18500	18000-21500	21000-24500	24000-27500	27000-30500	30000-33500	33000-35000
PCR forward primer	Ad7_5'-1	Ad7_5'-7	5-12	Ad7_5'-13	5-16	Ad7_5'-19	Ad7_5'-26	Ad7_5'-32	Ad7_5'-38	Ad7_5'-44	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68
PCR reverse primer	Ad7_3'-66	Ad7_3'-60	3-57	Ad7_3'-54	3-53	Ad7_3'-47	Ad7_3'-41	Ad7_3'-35	Ad7_3'-29	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	Ad7_3'-5	Ad7_3'-1
Sequencing primer	Ad7_5'-1	Ad7_5'-7	Ad7_5'-12	Ad7_5'-13	Ad7_5'-16	Ad7_5'-19	Ad7_5'-26	Ad7_5'-32	Ad7_5'-38	Ad7_5'-44	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68
	Ad7_5'-2	Ad7_5'-8	Ad7_5'-13	Ad7_5'-14	Ad7_5'-17	Ad7_5'-20	Ad7_5'-27	Ad7_5'-33	Ad7_5'-39	Ad7_5'-45	Ad7_5'-51	Ad7_5'-57	Ad7_5'-63	Ad7_5'-69
	Ad7_5'-3	Ad7_5'-9	Ad7_5'-14	Ad7_5'-15	Ad7_5'-18	Ad7_5'-21	Ad7_5'-28	Ad7_5'-34	Ad7_5_39_2	Ad7_5'-46	Ad7_5'-52	Ad7_5'-58	Ad7_5'-64	Ad7_5'-70
	Ad7_5'-4	Ad7_5'-10	Ad7_5'-15	Ad7_5'-16	Ad7_5'-19	Ad7_5'-22	Ad7_5'-29	Ad7_5'-35	Ad7_5'-40	Ad7_5'-47	Ad7_5'-53	Ad7_5'-59	Ad7_5'-65	Ad7_5'-71
	Ad7_5'-5	Ad7_5'-11	Ad7_5'-16	Ad7_5'-17	Ad7_5'-20	Ad7_5'-23	Ad7_5'-30	Ad7_5'-36	Ad7_5_40_2	Ad7_5'-48	Ad7_5'-54	Ad7_5'-60	Ad7_5'-66	Ad7_5'-72
	Ad7_5'-6	Ad7_5'-12	Ad7_3'-57	Ad7_5'-18	Ad7_5_P7900	Ad7_5'-24	Ad7_5'-31	Ad7_5'-37	Ad7_5'-41	Ad7_5'-49	Ad7_5'-55	Ad7_5'-61	Ad7_5'-67	Ad7_3'-1
	Ad7_5'-7	Ad7_5'-13	Ad7_3'-58	Ad7_5'-19	Ad7_3'-53	Ad7_5'-25	Ad7_5'-32	Ad7_5'-38	Ad7_5'-42	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68	Ad7_3'-2
	Ad7_3'-66	Ad7_3'-60	Ad7_3'-59	Ad7_3'-54	Ad7_3'-54	Ad7_5'-26	Ad7_3'-41	Ad7_3'-35	Ad7_5'-43	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	Ad7_3'-5	Ad7_3'-3
	Ad7_3'-67	Ad7_3'-61	Ad7_3'-60	Ad7_3'-55	Ad7_3'-55	Ad7_3'-47	Ad7_3'-42	Ad7_3'-36	Ad7_5'-44	Ad7_3'-24	Ad7_3'-18	Ad7_3'-12	Ad7_3'-6	Ad7_3'-4
	Ad7_3'-68	Ad7_3'-62	Ad7_3'-61	Ad7_3'-56	Ad7_3'-56	Ad7_3'-48	Ad7_3'-43	Ad7_3'-37	Ad7_3'-29	Ad7_3'-25	Ad7_3'-19	Ad7_3'-13	Ad7_3'-7	Ad7_3'-5

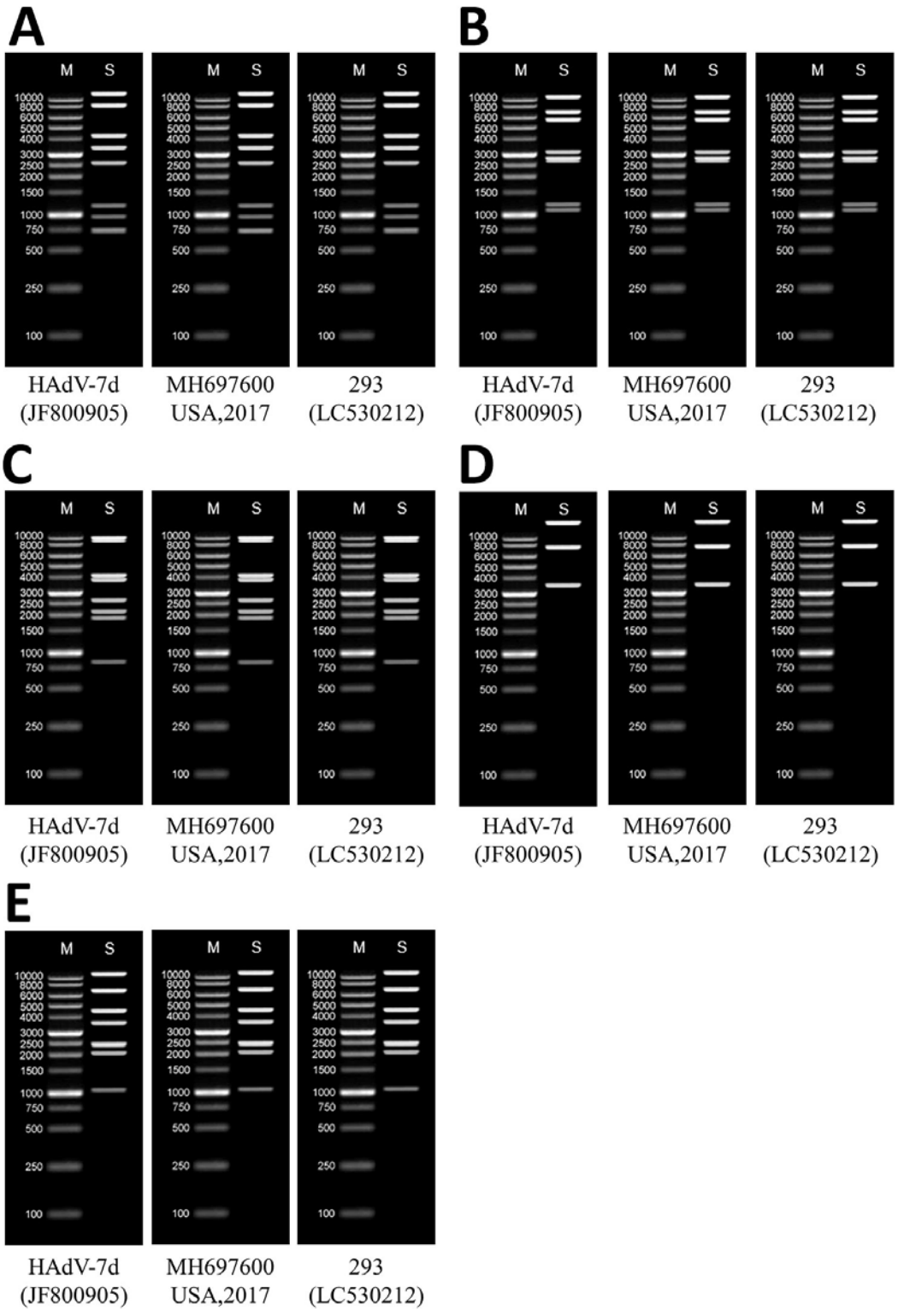
Fragment	1-3500	3000-6500	5500-8000	6000-9500	7500-10000	9000-12500	12000-15500	15000-18500	18000-21500	21000-24500	24000-27500	27000-30500	30000-33500	33000-35000
	Ad7_3'-69	Ad7_3'-63		Ad7_3'-57	Ad7_3'-57	Ad7_3'-49	Ad7_3'-44	Ad7_3'-38	Ad7_3'-30	Ad7_3'-26	Ad7_3'-20	Ad7_3'-14	Ad7_3'-8	
	Ad7_3'-70	Ad7_3'-64		Ad7_3'-58	Ad7_3_P9070	Ad7_3'-50	Ad7_3'-45	Ad7_3'-39	Ad7_3'-31	Ad7_3'-27	Ad7_3'-21	Ad7_3'-15	Ad7_3'-9	
	Ad7_3'-71	Ad7_3'-65		Ad7_3'-59		Ad7_3'-51	Ad7_3'-46	Ad7_3'-40	Ad7_3'-32	Ad7_3'-28	Ad7_3'-22	Ad7_3'-16	Ad7_3'-10	
	Ad7_3'-72	Ad7_3'-66		Ad7_3'-60		Ad7_3'-52	Ad7_3'-47	Ad7_3'-41	Ad7_3'-33	Ad7_3'-29	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	
						Ad7_3'-53			Ad7_3_33_2					
						Ad7_3'-54			Ad7_3'-34					
									Ad7_3_34_2					
									Ad7_3'-35					

*PCR was performed with GXL enzyme [TAKARA BIO, Kyoto, Japan] as previously described (Biggs et al. [*14*]). DNA fragments was purified and performed sequencing with indicated primers by FASMAQ Company, http://fasmac.co.jp.



Appendix Figure 1. Molecular phylogenetic analysis of human adenovirus 293 strain isolated in this study, compared with other human adenovirus type 7 reference strains. We aligned hexon (A), fiber (B) and penton (C) open reading frames using ClustalW (http://www.clustal.org) in

MEGA version 7 (https://www.megasoftware.net). We inferred the evolutionary history by maximum-likelihood method based on the Kimura 2-parameter model (A,C) or Tamura 3-parameter model (B). The tree with the highest log likelihood (A: −4462.55, B:-1392.74 and C: −2533.45) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. We obtained initial trees for the heuristic search automatically by applying neighbor-join and BioNJ (http://bionj.org) algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in number of substitutions per site. The analysis involved 12 nt sequences. All positions containing gaps and missing data were eliminated. The final dataset included a total of 2,805 positions (A), 978 positions (B), and 1,635 positions (C). We conducted evolutionary analyses in MEGA7. Sequence names are derived from the GenBank accession number, geographic location, year of sample collection, and virus type.



Appendix Figure 2. In silico restriction enzyme cutting pattern. A–E indicate restriction enzyme names used in this analysis: BamHI (A), BclI (B), BstEII (C), HpaI (D), and SmaI (5). HAdV-7d

(JF800905) and MH697600 are reference strains of HAdV genome type 7d, and 293 (LC530212) is isolated strain in this study. In silico analysis with several enzyme were performed with whole-genome sequences of JF800905, MH697600 and LC530212 using online software (http://www.molbiotools.com/restrictionanalyzer.html).